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(71)(72) Applicant and Inventor: HILLIKER, Sandra, R. [US/US]; 6756 Ranier Court, Riverside, CA 92506 (US).

(74) Agents: PARMELEE, Steven, W. et al.; Townsend and Townsend Khourie and Crew, One Market Plaza, 20th Fl., Steuart Street Tower, San Francisco, CA 94105 (US).

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(57) Abstract

This invention relates to human parathyroid hormone fragment in which the C-terminal amino acid is amino acid 35 to 38 and at least the first N-terminal amino acid has been removed or an analog or derivative thereof or a pharmaceutically acceptable salt or hydrolysable ester thereof and their use in treating bone degenerative diseases.

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PARATHYROID HORMONE FRAGMENTS AND ANALOGS

This invention relates to parathyroid hormone peptides and their use in treating bone disease. In particular, this invention relates to human parathyroid hormone peptide fragments and analogs which stimulate bone formation without the undesirable side effects associated with known parathyroid hormone peptides.

Degenerative bone disease, such as osteoporosis, is the most frequently occurring disease of the skeletal system. It is characterized by an imbalance in cell biological activity between bone formation and bone resorption which results in a reduction of bone mass. The problems involved in treating osteoporosis and conditions of reduced bone mass are largely unresolved. This is especially true in cases where a considerable reduction of bone mass has occurred and remodeling of the bone has decreased, as seen in the late stages of osteoporosis. Successful treatment should not only prevent progression of the disease, but should also stimulate new bone formation. Present treatment of osteoporosis includes hormone therapy, calcium and vitamin D supplements and sodium fluoride treatment, which for the most part are unsatisfactory. Bone loss may be slowed down by such treatment, but the total net gain in bone mass is limited.

It has been known for some time that the parathyroid hormone (PTH) regulates calcium and phosphorus metabolism and has a potent effect on bone metabolism. Administration of PTH however, induces a rapid proliferation of osteoclast cells associated with bone destruction, as well as a slower stimulation of osteoblast cells, which are necessary for bone formation. It is also well established that the first 34 N-terminal amino acids, (1-34)PTH, of the 84 amino acids, which make up PTH, are responsible for nearly all of the biological activity of the native hormone. Numerous clinical trials and experimental work have shown that both PTH and (1-34)PTH are only of very limited clinical value in treating osteoporosis. This is because of poor, inconsistent and unpredictable total net gain in bone mass, due to their stimulating osteoclastic activity as well as osteoblastic activity. In addition, continued use of PTH or (1-34) PTH induces tachiphylaxis within 12 to 18 months after treatment starts. Removal of the first one or two amino acids is known to reduce the activities of PTH and (1-34) PTH associated with stimulation of osteoclasts and bone resorption in vitro. It has been suggested that the parathyroid hormone fragment (3-34) PTH would stimulate osteoblast proliferation and bone formation without concurrent bone resorption. However, (3-34) PTH has antigenic activity and tachiphylaxis similar to (1-34) PTH, which cause undesirable antibody formation and resistance to the treatment. All of the presently known PTH fragments and analogs are of limited value in the treatment of osteoporosis, because of their bone resorption activity, antigenicity or tachiphylaxis.

It has now been found that human parathyroid hormone fragments, in which the C-terminal amino acid is amino acid 35 to 38, preferably 37 or 38 and at least the first N-terminal amino acid has been removed, and analogs and derivatives thereof stimulate osteoblast activity and maximize bone formation without undesirable levels of bone resorption, antibody formation, or tachiphylaxis. The human parathyroid hormone fragments of this invention can be represented in accordance with standard nomenclature by the formula (m-n)PTH, where m is the number of the first N-terminal amino acid and is at least 2 in the present invention and n is amino acid 35 to 38. In the preferred PTH fragments, analogs, and derivatives, m is 2 to 28 and comprise:

a) (3-38)PTH, (4-38)PTH, (5-38)PTH, (6-38)PTH, (7-38)PTH, (8-38)PTH, (9-38)PTH, (10-38)PTH, (11-38)PTH, (12-38)PTH. 13-38)PTH, (14-38)PTH, (15-38)PTH, (16-38)PTH, (17-38)PTH, (18-38)PTH, (19-38)PTH, (20-38)PTH, (21-38)PTH, (22-38)PTH, (23-38)PTH, (24-38)PTH, (25-38)PTH, (26-38)PTH, (27-38)PTH, or (28-38)PTH, especially (3-38)PTH;

b) (3-37)PTH, (4-37)PTH, (5-37)PTH, (6-37)PTH, (7-37)PTH, (8-37)PTH, (9-37)PTH, (10-37)PTH, (11-37)PTH, (12-37)PTH, (13-37)PTH, (14-37)PTH, (15-37)PTH, (16-37)PTH, (17-37)PTH, (18-37)PTH, (19-37)PTH, (20-37)PTH, (21-37)PTH, (22-37)PTH, (23-37)PTH, (24-37)PTH, (25-37)PTH, (26-37)PTH, (27-37)PTH, or (28-37)PTH, especially (3-37)PTH;

c) (2-35)PTH, (2-36)PTH, (2-37)PTH, or (2-38)PTH, especially (2-37)PTH and (2-38)PTH;

d) a [Tyr^{34}], [$Nle^{8,18}$ Tyr^{34}], [Phe^{23}], [Leu^{23}], [Nle^{23}], [Val^{23}], [Tyr^{23}], [alpha-naphthyalanine^{23}], or [beta-naphthyalanine^{23}] analog of a), b) or c); or

(e) a C-terminal amide derivative of a), b), c) or d), where PTH is human parathyroid hormone (hPTH), or a pharmaceutically acceptable salt or hydrolysable ester thereof.

The compounds of the invention are prepared by the known techniques, for example, the solid-phase peptide synthesis developed by Merrified ("Solid Phase Peptide Synthesis", Advances in Enzymology, 32:221-296, 1969). The C-terminal amino acid of the peptide is linked covalently via the carboxyl group to a solid support. The desired peptide sequence is prepared by stepwise coupling of single amino acids in protected form, if necessary, to a peptide chain growing from the carboxyl toward the amino terminus. Because each amino acid is coupled by nearly the same series of reactions, the need for elaborate strategies in the synthesis is minimized. The method is rapid; and after preparation of the desired peptide chain, the peptide can be easily deprotected by standard techniques, where necessary.

Alternatively, the peptide fragment can be prepared using recombinant DNA technology. The DNA for the desired peptide can be synthesized with a commercially available

oligonucleotide synthesizer (gene machine), such as Applied Biosystem Inc. automated synthesizer, using the manufacturer's procedure. The DNA is then expressed using a suitable vector in a conventional host, such as E coli, yeast or a mammalion cell to yield the desired peptide fragment.

The above parathyroid hormone peptides are useful in stimulating bone formation as indicated in male or female rats weighing more than 50 grams given doses of peptide between 100 ug/kg body weight and 1000 ug/kg body weight per day. The animals are divided into five groups of 12 animals each and given subcutaneous injections daily for up to one month. Group 1 is the placebo control; group 2 receives 100 ug/kg/day of the standard (1-38)hPTH; group 3 receives 100 ug/kg/day of peptide; group 4 receives 400 ug/kg/day of peptide; and group 5 receives 1000 ug/kg/day of peptide. For each group of animals, the levels of serum proteins and enzymes which reflect changes in bone metabolism are assayed at the end of treatment (e.g. serum osteocalcin, serum alkaline phosphatase, and serum tartrate resistant acid phosphatase). All animals receive tetracycline compounds during the last week of treatment: (1) either [$_3$H]-tetracycline at 25 uCi/100 g body weight to measure radioisotope incorporation into bone; or (2) two injections of tetracycline compounds - the first injection four to five days before sacrifice and the second injection one to two days before sacrifice - to fluorescently label newly mineralizing cancellous bone for the measurement of mineral apposition rate/bone formation rate by morphometry. Animals are sacrificed at the end of treatment and femurs, vertebrae, and

tibias collected for either radioisotopic or morphometric analysis of cancellous and/or cortical bone and for the analysis of bone extracts (e.g. alkaline phosphatase and tartrate resistant acid phosphatase).

The PTH hormone fragments, analogs, and derivatives of this invention are therefore useful in treating degenerative bone disease and provide a method of treating osteoporosis or hypercalcemia through the administration of a therapeutically effective amount of a parathyroid hormone peptide of the invention to a subject in need of said treatment. In addition, the invention provides a method of treating hyperparathyroidism, in particular, hyperparathyroidism expressed as a hypercalcemic crisis, renal failure, or hypertension, which comprises administering to a subject in need of said treatment a therapeutically effective amount of a parathyroid hormone peptide of the invention. This invention further provides a method of treating the disease state produced by a tumor or other aberrant cell overproducing a peptide hormone-like molecule and a method of treating immune diseases wherein the disease state comprises inflammation, an allergic response, or hyperactive lymphocytes comprising the administration of a therapeutically effective amount of a peptide hormone fragment, analog, or derivative of the present invention to a subject in need of said treatment.

The amount of parathyroid hormone peptide of the invention administered for the above uses will vary depending on the peptide used and the subject undergoing treatment. However, satisfactory results are obtained when the peptide

is administered daily or intermittently, and, if desirable, in 4-week or 6-week cycles followed by peptide free periods of 1 to 4 weeks. The peptides of the invention may be administered, in particular, to osteoporotic subjects or those with reduced bone mass, e.g., humans, in a dosage range of 20-2000 µg/day, preferably 100-1000 µg/day. It may be administered in a single dose or in several doses over 24 hours or as a continuous intravenous infusion. During and after therapy all parameters of calcium-metabolism which may be influenced by the parathyroid peptide should be determined. Thus bone, intestine, and kidney calcium metabolism as well as 1,25-$(OH)_2$-vitamin D synthesis should be monitored to determine the effectiveness of the therapy and possible metabolic side effects.

The peptides of the invention are administered enterally, parenterally, or subcutaneously admixed with conventional pharmaceutical carriers. They may be administered orally in the form of tablets or capsules or parenterally as solutions, e.g., sterile injectable solution, suspensions, e.g., aqueous suspension, or in depot form, in which the active ingredient is coated by known techniques to delay disintegration and absorption and thereby provide a sustained action over large periods of time. They may also be administered nasally in the form of sprays or rectally in the form of suppositories or enemas. They are preferably administered subcutaneously or rectally and especially nasally, since they do not stimulate bone resorption and cause erosion of the nasal cartilage. The smaller PTH fragments in which m is 18 to 28 are particularly useful for nasal administration, because they are more readily absorbed

through the nasal membrane. The pharmaceutical compositions of the invention are formulated as disclosed in the art and may contain up to about 90% of the active ingredients in combination with the carrier or adjuvant.

Example 1
Synthesis of (3-38)PTH

The synthesis of parathyroid hormone fragment (3-38) PTH is carried out by the solid phase method of Merrifield using a peptide synthesizer. Tertiary butyloxycarbonyl (Boc) group is used to protect the alpha-amino group of each amino acid during coupling. Other functional side groups are protected as follows: (a) the hydroxyl group of serine is protected as the O-benzyl ether; (b) the hydroxyl group of tyrosine as the O-2,6-dichlorobenzyl ether or p-bromobenzyloxycarbonyl ester; (c) the carboxyl group of glutamic and aspartic acid as the benzyl or cyclohexyl ester; (d) the imidazole nitrogen of histidine by the benzyloxymethyl (BOM) group; (e) the guanidine function of arginine by the p-toluenesulfonyl group and; f) the indole imine of tryptophane by the formyl group. The peptide-resin synthesis is carried out using the synthesizer manufacturer's specified protocols. After synthesis is complete, the peptide is deprotected, cleaved from the copolymer resin, and purified in accordance with the manufacturer's protocol to yield (3-38)PTH.

Following the above procedure (2-35)PTH, (2-36)PTH, (2-37)PTH, (2-38)PTH, (4-38)PTH, (5-38)PTH, (6-38)PTH, (7-38)PTH, (8-38)PTH, (9-38)PTH, (10-38)PTH, (11-38)PTH, (12-38)PTH, (13-38)PTH, (14-38)PTH, (15-38)PTH, (16-38)PTH, (17-38)PTH, (18-38)PTH, (19-38)PTH, (20-38)PTH, (21-38)PTH, (22-38)PTH, (23-38)PTH, (24-38)PTH, (25-38)PTH, (26-38)PTH, (27-38)PTH, (28-38)PTH, (3-37)PTH, (4-37)PTH, (5-37)PTH, (6-37)PTH, (7-37)PTH, (8-37)PTH, (9-37)PTH, (10-37)PTH, (11-37)PTH, (12-37)PTH, (13-37)PTH, (14-37)PTH, (15-37)PTH, (16-37)PTH, (17-37)PTH, (18-37)PTH, (19-37)PTH, (20-37)PTH, (21-37)PTH, (22-37)PTH, (23-37)PTH, (24-37)PTH, (25-37)PTH, (26-37)PTH, (27-37)PTH, or (28-37)PTH are similarly sythesized.

What is claimed is:

1. A human parathyroid hormone fragment in which the C-terminal amino acid is amino acid 35 to 38 and at least the first N-terminal amino acid has been removed or an analog or derivative thereof or a pharmaceutically acceptable salt or hydrolysable ester thereof.

2. A human parathyroid hormone fragment according to claim 1, which is (m-n)PTH, where m is 2 to 28 and n is 35 to 38, or an analog or derivative thereof or a pharmaceutically acceptable salt or hydrolysable ester thereof.

3. A human parathyroid hormone fragment according to claim 1, which is (m-n)PTH, where m is 2 to 28 and n is 37 or 38, or an analog or derivative thereof or a pharmaceutically acceptable salt or hydrolysable ester thereof.

4. An analog according to claim 2, which is the [Tyr^{34}], [$Nle^{8,18}$ Tyr^{34}], [Phe^{23}], [Leu^{23}], [Nle^{23}], [Val^{23}], [Tyr^{23}], [alpha-naphthylalanine^{23}] or [beta-naphthylalanine^{23}] analog of (m-n)PTH or a derivative thereof or a pharmaceutically acceptable salt or hydrolysable ester thereof.

5. A derivative according to claim 2, which is the C-terminal amide derivative of (m-n)PTH or an analog thereof or a pharmaceutically acceptable salt or hydrolysable ester thereof.

6. The human parathyroid hormone fragment according to claim 1, which is (2-38)PTH.

7. The human parathyroid hormone fragment according to claim 1, which is (3-38)PTH.

8. The human parathyroid hormone fragment according to claim 1, which is (2-35)PTH.

9. The human parathyroid hormone fragment according to claim 1, which is (2-36)PTH.

10. The human parathyroid hormone fragments according to claim 1, which is (2-37)PTH.

11. The human parathyroid hormone fragment according to claim 1, which is (3-37)PTH.

12. The human parathyroid hormone fragment according to claim 19, which is (4-38)PTH, (5-38)PTH, (6-38)PTH, (7-38)PTH, (8-38)PTH, (9-38)PTH, (10-38)PTH, (11-38)PTH, (12-38)PTH, (13-38)PTH, (14-38)PTH, (15-38)PTH, (16-38)PTH, (17-38)PTH, (18-38)PTH, (19-38)PTH, (20-38)PTH, (21-38)PTH, (22-38)PTH, (23-38)PTH, (24-38)PTH, (25-38)PTH, (26-38)PTH, (27-38)PTH, or (28-38)PTH.

13. The human parathyroid hormone fragment according to claim 1, which is (4-37)PTH, (5-37)PTH, (6-37)PTH, (7-37)PTH, (8-37)PTH, (9-37)PTH, (10-37)PTH, (11-37)PTH, (12-37)PTH, (13-37)PTH, (14-37)PTH, (15-37)PTH, (16-37)PTH,

(17-37)PTH, (18-37)PTH, (19-37)PTH, (20-37)PTH, (21-37)PTH, (22-37)PTH, (23-37)PTH, (24-37)PTH, (25-37)PTH, (26-37)PTH, (27-37)PTH, or (28-37)PTH.

14. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 1 and a pharmaceutically acceptable carrier therefor.

15. A pharmaceutical composition for nasal administration comprising a therapeutically effective amount of a compound according to claim 2 in which m is 18 to 28 and n is 35 to 38 and a pharmaceutically acceptable nasal carrier therefor.

16. A pharmaceutical composition for nasal administration comprising a therapeutically effective amount of a compound according to claim 2 in which m is 18 to 28 and n is 37 or 38 and a pharmaceutically acceptable nasal carrier therefor.

17. A method of treating bone degeneration in a subject in need of said treatment, which comprises administering to the subject an anti-bone degeneration effective amount of a compound according to claim 1.

18. A method of stimulating bone formation in a subject in need of said treatment, which comprises administering to the subject an amount of a compound according to claim 1 effective for stimulating bone formation.

19. A method of treating osteoporosis in a subject in need of said treatment, which comprises administering to the subject an amount of a compound according to claim 1 effective for the treatment of osteoporosis.

20. A method of treating osteoporosis in a subject in need of said treatment, which comprises nasally administering to the subject an amount effective for the treatment of osteoporosis of a compound according to claim 2 in which m is 18 to 28 and n is 35 to 38.

21. A method of treating osteoporosis in a subject in need of said treatment, which comprises nasally administering to the subject an amount effective for the treatment of osteoporosis of a compound according to claim 2 in which m is 18 to 28 and n is 37 or 38.

22. A method of treating hypercalcemia, hyperparathyroidism, a disease state caused by a tumor or an aberrant cell overproducing a peptide hormone, or an immune disease in a subject in need of said treatment, which comprises administering to the subject a therapeutically amount of a compound according to claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07375

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02, 37/24; C07K 5/00, 7/10, 15/00
US CL :530/324, 326, 327; 514/12, 13, 21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 326, 327; 514/12, 13, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS PTH and fragments or analog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A 4,086,196 (Tregear) 25 April 1978, col., 1, lines 40-53.	1-22
Y	US, A, 4,968,669 (Rosenblatt et al) 06 November 1990, col. 2, lines 35-63, col. 4, lines 1-3 and col. 5, lines 22-30.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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